Synopsis

In vitro enhancement of watermelon plant immunity by using some elicitors against gummy stem blight disease

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STATE OF THE ART

Citrullus lanatus (Thunb.) Matsum. & Nakai (watermelon) is a xerophytic crop, which belongs to the Genus *Citrulli* and an important member of family Cucurbitaceae [1]. It is widely cultivated throughout the worldwide such as tropical, semitropical and rigid regions [2]. Watermelon is abundant in essential nutritional elements such as carbohydrates, vitamins, lycopene, as well as amino acids including Arginine, citrulline, and Glutathione and these elements contribute positively to our cardiovascular health [3,4]. In 2021, watermelon, an economically significant fruit worldwide, had a total production of 101.6 million tonnes, with China as the leading producer, accounting for nearly half of the global output, followed by Turkey, Iran, and the USA ranking 2nd to 4th, each with annual production ranging from 2 to 3 million tonnes [5]. In 2021-22, India secured the 9th position in watermelon production with a total annual yield of 3,225.44 tonnes, wherein Uttar Pradesh contributed the highest share at approximately 706.65 tonnes, followed by Andhra Pradesh, Tamil Nadu, and Karnataka, ranking 2nd to 4th, with respective annual productions of 628.57, 315.19, and 260.90 tonnes, while Jharkhand stood at the 10th position with an annual production of 74.42 tonnes [6]. The Cucurbits plants are affected by various stresses like biotic stress (Fungi, Bacteria Virus etc.) and abiotic stress (Cold, Heat, Salt, Drought etc.) during the growth and development of plant [7]. Whereas, one of the fungi *Stagonosporopsis* species (*S. Cucurbitacearum*, *S. Citrulli* and *S. Caricae*) causes gummy stem blight (GSB) disease in the watermelon plant resulting in significant yield and quality losses of fruit and vegetables up to 43 % [8-13]. GSB are known to found in every place where cucurbits are cultivated, 37 species of cucurbits from 22 genera are known to susceptible to Gummy stem blight [14-16]. Gummy stem blight (GSB) manifests with circular, tan to brown water-soaked lesions on various plant parts, affecting watermelon with symptoms such as crown blight, stem cankers, and extensive defoliation, leading to substantial economic losses for growers, and chemical control methods often pose environmental health risks (Chiu and walker, 1949). To control the gummy stem blight, several fungicides are used such as azoxystrobin, moncozeb, cyprodinil , fludioxonil , difenoconazole and chlorothalonil [17,18]. But these fungicides affect human health as well as to the environment and also highly cost effective [19-21]. Several approaches have been adopted to develop resistance plants against GSB disease such as breeding approaches, transgenic approaches, biological approaches and by enhancement of immunity of plant by using elicitor applied by seed treatment, foliar spray and soil drenching, no effective watermelon varieties have been developed against this fungal disease [22-24] . Recently, CRISPR Cas9 mediated gene editing and RNAi techniques were employed to

develop resistant various plant against fungal diseases through host-induced gene silencing and nanoparticles-mediated genome editing [25-30]. Lots of studies on Genetic resistance sources have been reported against GSB, but no resistance cultivars have been released commercially [20,31]. So, in the current study we have approached to develop highly immunized *in vitro* watermelon plant by using some elicitor.

PROBLEM TO BE INVESTIGATED

Gummy stem blight is one of the devastating fungal diseases caused by *Stagonosporopsis species* and affect during the growth and development of watermelon plants as well as yield loss up to 43%. To control this fungal disease several chemicals has been used, but these chemical effect to the human health as well as toS the environment, also till date there is no effective way has been reported to control this disease. So, the objective of this study is to enhance the immunity of watermelon plant against *Stagonosporopsis species* fungus causing gummy stem blight disease by using some elicitors and assessment of enhanced watermelon plant immunity against fungal pathogens.

OBJECTIVE OF THE INVESTIGATION & MOTIVATION

1. Standardization of regeneration protocols of watermelon plant.
2. *In vitro* enhancement of watermelon plant immunity by using some elicitors.
3. Assessment of *in vitro* enhanced immune watermelon plant against Gummy Stem Blight disease.

HYPOTHESIS

Watermelon plant is affected by fungal disease which is caused by *Stagonosporopsis species* and it causes yield losses up to 43%. So, in the current study we hypothesize that to control the fungal disease by enhancement of plant immunity by using some elicitors (chitosan, salicylic acid, chitin etc.). After increasing the plant immunity, we have to bioassay against this fungal pathogen to check the control of disease.

RESEARCH DESIGN AND METHODOLOGY

A. Materials

1. **Plant and fungal material:** Seeds of Watermelon (*Citrullus lanatus*) plant and fungal species (*stagnosporopsis species*) will be obtained from the collection centre.
2. **General chemicals:** MS media, Phyto hormones, ethanol, distilled water, Sabouraud dextrose agar, potato dextrose media, elicitors.

3. Tools to be used:

Equipment: Glassware, PH meter, Autoclave, Measuring cylinder, Culture tube, Weighing machine, Pipette, Spatula, Laminar Air Flow, Petri plates, Forceps, Burner, Incubator, Haemocytometer.

B. Methodology

1. **Standardization of regeneration protocol of watermelon plant:** For *in vitro* multiplication, we will follow the regeneration protocol of Mahto et al. [32] with slight modification. Develop a highly efficient regeneration protocol for watermelon by using different explants. For the regeneration we have to optimize a different phytohormones combination and concentration in plant growth medium and the culture conditions like light and temperature.
2. **Enhancement of immunity of watermelon by using different elicitor during *in vitro* propagation of watermelon**.
3. **Assessment of enhanced immune watermelon plant for their efficacy against gummy stem blight disease.**
    - Collection of spores
    - Fungal challenge assay on enhanced immune watermelon plant
    - Microscopy of infected plant
4. **Data Analysis:** During the investigation all data will be analysed by using statistical tool for the significant differences.

SIGNIFICANCE OF THE STUDY

Watermelon is an economically important cucurbits crop worldwide with total production per annual is 101 million tonnes across the world. It is good source of soluble fiber that promotes cardiovascular health and reduce the risk of heart diseases. It is also rich in potassium and vitamins. Despite of the total production of watermelon, million tonnes of fruits approximately up to 43 % are lost in fields due to Gummy stem blight. In the current study, we aim to find out the solution by increasing the immunity of watermelon against gummy stem blight by using some elicitor. It will help to reduce frequent uses of fungicides and to control gummy stem blight that leads to negligible impact on environment as well as human health and it will also help the cultivators via reduction in losses of yield by gummy stem blight and cultivators also get benefits economically.

IT`S RELEVANCE TO THE PRESENT DAY PROBLEM AND NEED OF THE SOCIETY

Plants are affected by various pathogens which results in decrease in the yield and due to this Cultivators have to face significant economic losses, to reduce the losses, cultivators mainly depends on harmful chemicals like fungicides, insecticides, pesticides etc. that has a negative impact on environment as well as human health. To reduce high dependency on chemicals for managing the plant disease and to save environment from damages, it is necessary to use methods like gene editing technology, immunity enhancement technology and nanotechnology against management of diseases and there might be less negative effects by using these techniques.

GSB is a fungal disease and it is pathogenic to cucurbits like melon, cantaloupe, cucumber, watermelon etc. And causes drastic losses in yield and also affect economically.

So, the current study will benefit the society by leading to the discovery of highly immunized watermelon plant against GSB by using elicitor.

CONTRIBUTION TO THE EXISTING KNOWLEDGE

Currently no resistant line against GSB have been available commercially. Enhancement of immunity of watermelon against gummy stem blight will reduce the heavy dependency on fungicides and reduce the yield losses which will economically benefit to cultivators and also protect the environment by the negative effect of using fungicide.

FUTURE PERSPECTIVE

As such, the suggested study will deal with the enhancement of immunity of watermelon by using elicitor against GSB and Evaluation of enhanced immune watermelon plant against GSB. This technique will also use to control other diseases against various pathogen in different plant species.

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